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Phytochemical Investigation on Isolation and Characterization of 2 Hydroxy-1, 4-Naphthoquinone (*Lawsonia Inermis L.*) from the Powdered Leaves of Henna Plant

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Abstract
Natural products are typically secondary metabolites, produced by plants and microorganisms in response to external stimuli such as nutritional changes. They are widely recognized in the pharmaceutical industry for their remarkable structural diversity and wide range of pharmacological activities[1]. Henna is a Persian word, which describes a small flowering shrub (Lawsoniainermis, also called mignonette tree) is a flowering plant which has been used since the Bronze Age to dye skin (including body art), hair, fingernails, leather, silk and wool. In several parts of the world it is traditionally used in various festivals and celebrations. The name is also used for dye preparations derived from the plant. Binomial name; Lawsoniainermis L. The trivial name of this compound was hennotannic acid. Interesting from the chemists' point of view are the redox properties of naphthoquinones such as lawsone[1]. The henna plant is a tall flowering shrub or tree about 5 m in height, native to tropical and subtropical regions of Africa, southern Asia, and northern Australia in semi-arid zone and oases in the Sahara[1]. Henna shrubs need light and warmth and are regarded as rather pest resistant. It is glabrous; multi branched with spine tipped branch lets. Leaves are opposite, entire, glabrous, sub-sessile, elliptical, and broadly lanceolate (1.5–5.0 cm x 0.5–2 cm), acuminate, having depressed veins on the dorsal surface[1]. Henna is commercially cultivated in UAE, Morocco, Yemen, Tunisia, Libya, Saudi Arabia, Egypt, western India, Iran, Pakistan, Bangladesh, Afghanistan, Turkey, Somalia and Sudan. Presently the Pali district of Rajasthan is the most heavily cultivated henna production area in India, with over 100 henna processors operating in Sojat City[1]. Use of henna for body art has enjoyed a recent renaissance due to improvements in cultivation, processing, and the emigration of people from traditional henna-using regions for skin dyeing; a paste of ground henna (either prepared from a dried powder or from fresh ground leaves) is placed in contact with the skin from a few hours to overnight. Henna stains can last a few days to a month depending on the quality of the paste, individual skin type, and how long the paste is allowed to stay on the skin[1]. Henna also acts as an anti-fungal and a preservative for leather and cloth. Henna flowers have been used to create perfume since ancient times, and henna perfume is experiencing resurgence. Henna repels some insect pests and mildew. Henna's coloring properties are due to lawsone, a burgundy organic compound that has an affinity for bonding with protein. Lawsone is primarily concentrated in the leaves, especially in the petioles of the leaf. Henna will not stain skin until the lawsone molecules are made available (released) from the henna leaf. Fresh henna leaves will stain the skin if they are smashed with a mildly acidic liquid. The lawsone will gradually migrate from the henna paste into the outer layer of the skin and bind to the proteins in it, creating a fast stain[1]. Henna stains are orange soon after application, but darken over the following three days to a reddish brown. Soles and palms have the thickest layer of skin and so take up the most lawsone, and take it to the greatest depth, so that hands and feet will have the darkest and most long-lasting stains. Steaming or warming the henna pattern will darken the stain, either during the time the paste is still on the skin or after the paste has been removed. Chlorinated water and soaps may spoil the darkening process: alkaline products may hasten the darkening process[1].

Keywords: *Phytochemical, Isolation Characterization, 2Hydroxy-1, 4-Naphthoquinone, Powdered Leaves, Henna Plant*

1.0 INTRODUCTION

World is endowed with a rich wealth of medicinal plants. Man cannot survive on this earth for long life without the plant kingdom because the plant products and their active constituents played an important role. There is a widespread belief that green medicines are healthier and more harmless or safer than synthetic ones. Medicinal plants have been used to cure a number of diseases. Though the recovery is slow, the therapeutic use of medicinal plant is becoming popular because of its inability to cause side effects and antibiotic resistant microorganisms[1]. Seeking healing by using plants is an ancient practice. Various cultures applied poultices and imbibed infusions of hundreds, if not thousands of indigenous plants dating back to prehistory. The development of new antimicrobial agents is a research area of the utmost importance.

The present attempt is to review and compile updated information on various aspects of *Lawsoniainermis* (Linn), a plant used all over the world. This plant is commonly known as Henna or Mhendiand abundantly available

in tropical and subtropical areas. Ancient history of India describes itsdiverse uses and also plays appreciable role in Ayurvedic or natural herbal medicines. Henna has beenused cosmetically and medicinally for over 9,000 years. Traditionally in India, henna is applied tohands and feet. Henna symbolizes fertility. Its use became popular in India because of its coolingeffect in the hot Indian summers. Henna leaves, flowers, seeds, stem bark and roots are used intraditional medicine to treat a variety of ailments as rheumatoid arthritis, headache, ulcers, diarrhoea,leprosy, fever, leucorrhoea, diabetes, cardiac disease, hepatoprotective and colouring agent[1].

Lawsoniainermis L is a much branched glabrous shrub or small tree, cultivated for its leaves although stem bark, roots, flowers and seeds have also been used in traditional medicine. The plant is reported to contain carbohydrates, proteins, flavonoids, tannins and phenolic compounds, alkaloids, terpenoids, quinones, Coumarins, xanthones and fatty acids. The plant has been reported to have analgesic, hypoglycemic, hepatoprotective, immunostimulant, anti-inflammatory, antibacterial, antimicrobial, antifungal, antiviral, antiparasitic, antitrypanosomal, antidermatophytic, antioxidant, antifertility, tuberculostatic and anticancer properties. It is now considered as a valuable source of unique natural products for development of medicines against various diseases and also for the development of industrial products. This review gives a bird's eye view mainly on the traditional uses, Phytochemistry and pharmacological actions of the plant[1].

Para Phenylenediamine(PPD), a derivative of para-nitroaniline is widely used in hair dye formulations, in dyeing furs and in photochemical industries. It has also been used to intensify the color of henna and to accelerate the dyeing process. Accidental or deliberate ingestion of henna containing PPD has a high mortality rate (up to 31%) owing to rhabdomyolysis and renal failure. We report a case of systemic poisining with henna for suicidal intent. The characteristic features of intoxication are eczematous dermatidis, erythememultiforme eruptions, angioneurotic edema, rhabdomyolysis and acute renal failure

2.0 LITERATURE REVIEW

Henna has been in use for centuries in various parts of world. Even as far back as 5000 years ago. Henna was used for coloring the hair and nails of Mummies. In the Near East it is planted as a wind break for vineyards and melon plantations for it forms a bushy leafy plant. The flowering, twiggy stems were woven into chaplets by Egyptian maidens and to this day, sprays of blossom are sold in the streets of Cairo and Damascus with the cry "oh odor of Paradise: of flowers of henna." Mohammed is said to have dyed his beard with henna and Mohammedan women use it to color their hair with a reddish tint or mixed with indigo ('reng'), it imparts a fine blue-black gloss to beard and hair and was made into a paste to apply to the body especially among certain orthodox Muslim and Hindu women for the bride's hands and feet on the eve of her wedding. Henna leaves were used by the people of ancient civilizations to dye the manes and tails of their horses. The Egyptians are said to have prepared both oil and an ointment from the flowers for making the limbs supple. Sleeping on a pillow stuffed with henna flowers is considered to have a soporific effect on patients suffering from sleeplessness.

2.1 Scientific Classification

Kingdom : Plantae
Subkingdom :Tracheobionta
Division :Magnoliophyta
Class :Magnoliopsida
Order :Myrtales
Family :Lythraceae
Genus :*Lawsonia*
Species :*L.inermis*L.

2.2 Geographical Distribution

During the last twenty years, henna body art has emerged from South Asia, the Levant, the Arabian Peninsula and North Africa into the popular culture of the USA, Canada, Europe and the UK. The western world has little understanding of henna, its techniques, traditions or history, and no legal or commercial framework for definition or regulation of henna. There have been scattered mentions of henna in anthropological, botanical, medical, historical, economic and legal literatures, but there has never been an integrated multidisciplinary study of what henna is. There is no resource on henna that includes where it was used, when it was used, how it was used, why it was used, who used it, nor have these elements been linked. The lack of a coordinated source of information about henna hampers not only academic for discussion about henna's history and traditions, but stands in the way of ordinary people's understanding and enjoyment of henna.The western academic community has paid scant attention to henna. As a women's tradition, it was not easily available to male anthropologists and explorers, nor was body art studied seriously

as a cultural expression until recently. There are well-documented henna traditions among Armenian Christians, Coptic Christians, and among all of the Jewish groups that lived North Africa, the Arabian Peninsula and the Levant.

The scarcity of reliable information on henna in western publications adversely impacts legal, economic, health, cultural, and religious issues tangent to henna. A fundamental academic investigation of henna, enabled by defining what henna is and is not, is sorely needed to integrate it into the body of knowledge, and to facilitate henna's introduction into western culture. Up to this point, there has been no organized study of henna, not even a set of criteria for undertaking that study. A mature henna tree produces five to seven kilos of henna leaves per year. The first henna plants were introduced into the Caribbean through Indian laborers working in the British sugar industry in Trinidad between 1845 and 1917 where the plants were locally known as "Jamaican Mignonette". These were cultivated as a hedge plant and appreciated for the flowers, but body art use did not extend beyond the Indian immigrant community. Other henna-using immigrant communities in the Americas had to import henna where it could not be grown locally, or go without Henna powder becomes stale and loses dye content in about three months unless it is packaged in airtight, climate-controlled packaging. When dried henna leaves are powdered, the lawsone degrades in contact with air or light. Henna kept in loose, porous packaging for more than three months makes pale orange stains, or at one year, little or no stain at all. If henna is packed as dried whole leaves in dark, moisture proof containers, it has a shelf life of six months to a year.

Prior to the 1990's henna was packaged in cloth bags, cardboard and cellophane packaging.
This degradation has always limited the geographic extent of henna material culture. When pack animals were the normal transport, traveling henna at 25 km per day, and cloth sacks were the normal packaging, one would expect henna customs to be confined to 1500 km from the henna-growing zone. Henna traveling for more than three months, then brought to market for resale, would be in demise before it could be purchased and applied to hands or hair.Henna grows best in tropical savannah and tropical arid zones, in latitudes between 15° and 25° N and S, and produces highest dye content in temperatures between 35 ^{o}C and 45^{o}C. It can also occupy frost-free Mediterranean scrub zones, though it doesn't develop maximum dye content without high summer heat. Optimal soil temperatures for germination are 25 – 30^{o}C. It does not thrive where minimum temperatures are below 11^{o}C. Temperatures below 5^{o}C will kill the henna plant. Henna grows easily and self-reproduces in alluvial soils along seasonal creeks or near water holes in tropical zone semi-arid and desert regions.

In Egyptian villages it is sometimes cultivated as a hedge plant, growing alongside rose bushes.Henna thrives in low-latitude semi-arid to arid zones, Pali district, the most intensive cultivation area of henna in the world, is on the fringes of the Thar Desert, and the rainfall was between 400 and 420 mm per annum at between 1968 and 2004. Marwar province, also a henna-growing region ranged from 460 to 500 m per annum in 1980 to 2004. Eighty five percent of the annual rain falls between August and September, with little rain between monsoons. These areas have sandy loam soil, and little ground water. The plants thrive in a region of chronic drought. In areas of high precipitation, or in heavy, damp soil, henna is vulnerable to scale insects, aphids, and root rot, and the dye content is lower than in areas with prolonged droughts.

2.3 Physical Characteristics of the Plant Lawsonia Inermis

The leaf of *Lawsoniainermis*L. is short, smooth, compound, ovate-lanceolate, acute, symmetrical, entire, pinnate, opposite, sweet smelling, characteristics or bitter in taste and varies in length, Lawsone is mainly present in the marginal vein or petiole in large quantity. The main constituents in the plant are carbohydrates, glycosides, tannins, phenolic compounds and gums and mucilage. Henna of commerce is the dried leaf of *Lawsoniainermis*L., a shrub or small tree which is indigenous to the area between Iran and northern India. The plant has been introduced widely throughout the tropics and sub-tropics as an ornamental (frequently as a fragrant hedge), for home use as a dyestuff and elsewhere as a commercial crop, notably in several North African countries. It is a tall shrub or small tree, 2.6 m high. It is glabrous, multibranched with spine tipped branchlets. Leaves are opposite, entire, glabrous, sub-sessile, elliptical, and broadly lanceolate (1.5–5.0 cm x 0.5– 2 cm), acuminate, having depressed veins on the dorsal surface. Henna flowers have four sepals and a 2 mm calyx tube with 3 mm spread lobes. Petals are obvate, white or red stamens inserted in pairs on the rim of the calyx tube. Ovary is four celled, style up to 5 mm long and erect. Fruits are small, brownish capsules, 4–8 mm in diameter, with 32–49 seeds per fruit, and open irregularly into four splits.

Aqueous extraction of the dried leaf provides a dye which can range in color from black, to red through to blonde (neutral). From ancient times, henna has been employed as a cosmetic dye for hair, skin and nails and it has acquired a particular significance in Islamic culture. More recently, there has been an increase in its usage as a hair dye in Western Europe and North America. Prior to the widespread availability of synthetic dyestuffs, henna was employed also as a dye for textiles and leather.The major pigment in henna leaf is lawsone (2-hydroxy-l,4-napthaquinone). This fixes strongly to protein and, consequently, it has fast-dyeing properties. Considerable variability can exist between lots of dried henna leaf in the pigment content (which normally ranges between 1 and 2%) and,

more importantly in cosmetic applications, in the color tone. International trade is conducted in whole or powdered leaf.

The leaf of *Lawsoniainermis*L. is short and smooth. The midrib is distinct from the lamina. It is broadly shallow on the adaxial side and convex on the abaxial side. It has a single layered polygonal epidermal cells containing cuticle on outer layer only. It also consists of unicellular covering trichome. Diacytic stomata are present on both the surface. The leaf of *Lawsoniainermis*L. is dorsiventral as oblong palisade cells are present below the upper epidermis and absent on lower epidermis. The abaxial epidermis is also very thin and distinct. The ground tissue of the midrib is parenchymatous and homogeneous. The cells are circular or angular and compact. Tannin is seen in some of the cells. The vascular strand is single, small, collateral and hemispherical in shape. It consists of a thick horizontal band of xylem and a fairly wide band of phloem. Xylem elements are narrow, angular, thin walled and somewhat diffuse.

2.3.1 Lamina

The lamina is uniformly flat with even surface. Both adaxial and abaxial epidermal layers are thin and distinct. The mesophyll tissue is differentiated into palisade and spongy parenchyma. It consists of about 4 to 6 layers of small, thin walled, vertically oblong and polyhedral parenchymatous cells. The cells are compact. Some of the cells are filled with dark tannin content. The vascular bundles of the lateral veins are small and not prominent. They are collateral with a thin adaxial patch of xylem and abaxial rest of phloem. The leaf margin is narrower than the middle part.

2.3.2 Epidermal Tissue

In paradermal section of the lamina, the epidermal layer appears as flat polygonal mat of cells which are variable in shape and size, they are polyhedral, and the anticlinals walls are thin, straight with the deposition of cuticle. Some of the epidermal cells are smaller and have dark tannin content. The stomata are present on both surfaces. The abaxial epidermal cells are larger and their walls are slightly thicker and wavy. Stomata are abundant. The stomata are Dicytic type. Each stoma is surrounded by two subsidiary cells the long axis of which is perpendicular to the long axis of stoma pore. The stomata are elliptical with wide opening.

2.3.3 Petiole

In cross sectional view, the outline of the petiole is elliptical with adaxial groove along with upper part and ovate, without adaxial grooves along the lower part of the petiole. The surface of the petiole is even and smooth. The epidermal layer is thin and very distinct. The ground tissue is homogeneous and parenchymatous, the cells are thin walled and compact. Some of them contain tannin.

2.4. The Henna Plant (Lawsonia Inermis)

Henna is the Semitic language word for the plant, *LawsoniaInermis*, the paste made of pulverized henna leaves, and the body art created with that henna paste. Henna contains a dye, Lawsone, or hennotannic acid, 2-hydroxy-1, 4-naphthoquinone that stains skin, nails and hair dark blood-red. Crushing fresh or dried henna leaves with lemon juice or some other acidic liquid makes henna paste. Henna paste is applied to skin, fingernails or hair. When the henna paste is left on for several hours, the keratin and collagen become thoroughly saturated, with Lawsone. When the paste is removed, an orange stain remains. This stain darkens to deep reddish brown over 48 hours.vA skilled henna artist can create complex patterns with shaded colors, and the results can look like dark lace. When the paste is left on longer, and under hotter conditions, such as at a women's bath, or hammam, stains are darker, and retain their vivid color longer. Henna stains usually last about three weeks. When henna is applied at the end of menstruation, the stain is generally bright colored through the time period of ovulation, and fades to vanishing by the onset of the next menstrual period. As the skin exfoliates and regenerates, the henna stained cells exfoliate, so the henna pattern disappears in about 3 weeks. As hair and fingernails grow out, the undyed roots show.

North African and Middle Eastern women stained and ornamented hands, feet, nails and hair with henna when they visited the *hamam*, a traditional women's public bath. This bath was required at the end of their menstrual cycle, though well-to-do women went more frequently. Henna was most commonly applied to fingertips and fingernails, staining them red or rust color to near black. Henna was also applied to the feet and soles, in patterns that resembled slippers. Applying henna to fingertips and nails prevented cuticles from splitting, and strengthened nails for rough village women's work. Applying henna to soles kept heels from cracking and relieved cuts and blisters from rough sandals or stony ground. Women believed henna purified them and protected them from disease. There may be some medical basis for these beliefs, as some studies have demonstrated henna deters some bacterial and fungal growth, and may have a localized analgesic effect.

In many communities, henna was used to deter malevolent spirits and the Evil Eye. When a woman felt vulnerable, or believed someone had cast the Evil Eye on her, she might hire a specialist to henna complex patterns on her skin at a henna party. North African women hennaed diamond-shaped *Khamsa*henna patterns to repel the evil eye, protect the wearer, and enhance their sexuality. An old Moroccan proverb stated that "A woman without henna is like wheat without salt," indicating that a woman is more "appetizing" when she has henna. Arab women used a combination of diamond patterns with written charms to protect the wearer as well as patterns intended to enhance beauty and eroticism. Sudanese women wear floral henna patterns to emphasize their femininity and to attract benevolent spirits. Women used henna because their health and fertility, and husband's love were never guaranteed, and they often wished to actively better their situation rather than passively accept "the will of God".

The Evil Eye was often considered the cause of lost fertility, lost love, lost health, and women often believed a rival wife or spirit had cast the evil eye. Persian women would henna intricate patterns that would entangle the Evil Eye so it would not touch their skin and penetrate their soul. Many believed that malevolent *jinn*, supernatural spirits were attracted to their menstrual blood, and that their evil intent could be thwarted by henna and protective patterns; *jinn* would "bounce off and shatter" if they touched star shaped henna patterns. Women soiled with reproductive blood were believed to be highly vulnerable to attack and possession by the Evil Eye and *jinn*, and henna was specifically believed to deter malevolent spirits and encourage benevolent spirits in Persia, Sudan, Morocco, and Mauritania. The roots of this plant are useful in burning sensation, leprosy, strangury, premature grey of hair and tuberculostatic activity. Recently, this compound has been reported to have a growth inhibitory effect against human colon carcinoma, HCT- 15 cells. There are three types of henna like Neutral henna, Red henna and Black henna. Neutral henna, a green powder that smells like freshly cut grass, is neither henna nor neutral. It is *Cassia obovata*, contains anthraquinones, particularly chrysophanic acid, a remarkable anti-fungal, anti-microbial and antibacterial compound. *Cassia obovata*does not color hair. Red henna, a green powder that smells like hay, is *Lawsoniainermis*, commonly known as henna. Henna will stain your hair red-orange; but this stain is translucent and will combine with your natural color. Black henna, a green powder that smells like frozen peas, is neither black nor henna. It is indigo, *Indigoferatinctoria.*

2.5 Occurance of Lawsonia Inermis L.

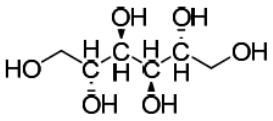
L. inermis is widely distributed throughout the Sahel and into Central Africa; it also occurs in the Middle East. It grows mainly along watercourses and in semi-arid regions and is adapted to a wide range of conditions. It can withstand low air humidity and drought. Henna requires high temperatures for germination, growth and development. The different species of *Lawsoniainermis*L. are *Lawsoniaalba*and *Lawsoniaspinoza/spinosa*. *Lawsoniaalba*and *Lawsoniaspinoza*are misleading older names for *LawsoniaInermis L*. When henna is a small and immature plant, it has low dye content and is spineless; when mature, it develops spines and higher dye content. Henna plants undergo this change when they are 3 years old. When western botanists saw juvenile and mature henna plants, they thought they were seeing two species, and gave them different botanical names. *Lawsonia*also has different colors of flowers. The plants varieties with white flowers are sometimes called *var. alba*, but they are used for dye as the plants with yellow, pink and red flowers. Henna is a juvenile plant for the first two years. The leaves do not have high lawsone content, and the branches do not have thorns. In mature plants, thorns develop at the leaf buds during dormant phases.

The henna fruits ripen at the end of the summer. In each henna fruit there is an average of 40–45 seeds. The leaves are generally useful in treatment of that troublesome and painful affection called as "burning of foot". For this purpose the fresh leaves are beaten into paste with vinegar or lime juice and applied as poultice to the soles of the feet. The flowers are intellect promoting cardio-tonic, refrigerant, febrifuge and tonic. The seeds are antipyretics, constipating, and are useful in intermittent fever, insanity, amentia, dysentery, diarrhea and gastropathy. The roots are bitter, diuretics, emmenogogue, abortifacient, are useful in dyspepsia, leprosy, skin disease, amenorrhea, dysmenorrhea, and premature graying of hairs. The leaves are useful in wounds, ulcers, cough, bronchitis, leucoderma, scabies, boil, hepatopathy, spleenopathy, ophthalmic conditions, falling of hairs and jaundice. Plant henna extract is used as hair growth stimulators for the treatment of dandruff and as hair colorant or dye. Mechanism of action appears to be done by acceleration of blood circulation, activation of dermal papilla and increase nutrition to the hair follicles. Mean annual temperature: 19-27^{o}C, Mean annual rainfall: 200-4200 mm, Soil type: Prefers sandy soils but can tolerate clays and poor, stony, sandy soils; optimum soil pH is 4.3-8.

2.6 Chemical Constituents

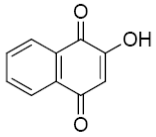
The active constituents of the leaf islawsone (0.5-1.0%). Other constituents are 5-10% gallic acid, white resin, tannin and xanthones are the other contents of the leaves. The **'Lawsone'** is principally responsible for the colourant property of the henna leaves**.** The chemical studies on the plant *L. alba* were first time undertaken in 1886 by M. A. Harraory, who reported the isolation of hennotannic acid, from the European specie, which was considered as the coloring agent of henna[14]. Later on, in 1920 Tommasi reported that the key coloring constituent of henna leaves is 2-

hydroxy-1, 4-naphthoquinone ($C_{10}H_6O_3$.)[15]. O. A. Oesterle in 1923 isolated crystals of D-mannitol from 98% ethanolic extract which were stable at room temperature[15].



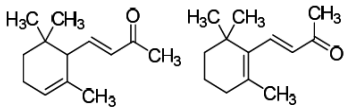
D-Mannitol

C. S. Anon in 1924 extracted crushed leaves of henna with warm or cold aqueous solution of an alkali earth salt (e.g. Ca and Mg salt from the Solvay ammonia-soda process) to give a coloring agent called lawsone. In 1928 another method was reported by Syed B.Ali who extracted lawsone with benzene from water extract. The extract was treated with Pb acetate and Pb was removed by H_2S.



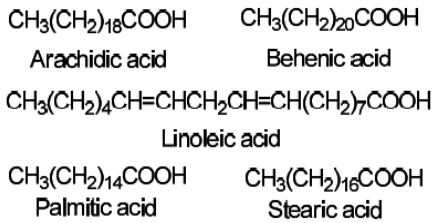
Lawsone
(2-Hydroxy-1,4-naphthoquinone)
(Principle active constituents of *Lawsonia* alba)

The structure of 2-hydroxy-α-naphthoquinone obtained by Lal and Dutt from Indian specie was confirmed by comparing its physical data with synthetically prepared 2-hydroxy-α-naphthoquinone and its derivatives[15].Anita and Kaushal in 1950 worked on henna flowers and confirmed the presence of α-ionone and β-ionone in essential oil, both having same mol: formula $C_{13}H_{20}O$.



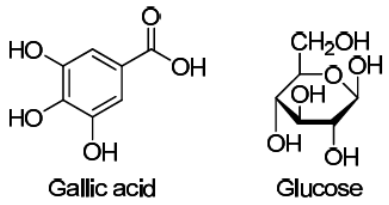
α-Ionone β-Ionone

S. R. Agarwal*et al*. in 1959 analyzed *L. alba*seed oils (10-11%) and determined its composition as arachidic acid, behenic acid, linoleic acid, palmatic acid and stearic acid[15].



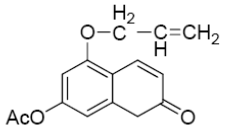
$CH_3(CH_2)_{18}COOH$ Arachidic acid
$CH_3(CH_2)_{20}COOH$ Behenic acid
$CH_3(CH_2)_4CH{=}CHCH_2CH{=}CH(CH_2)_7COOH$ Linoleic acid
$CH_3(CH_2)_{14}COOH$ Palmitic acid
$CH_3(CH_2)_{16}COOH$ Stearic acid

B. N. Sastri in 1962 reported gallic acid from the leaves and glucose from the whole plant of *Lawsonia inermis*[16].

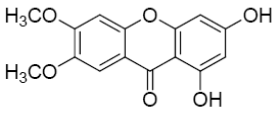


Gallic acid Glucose

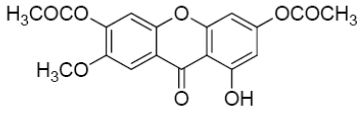
D. K. Bhardwaj*et al*. in 1976 reported a new coumarin, lacoumarin (5-allyloxy-7- acetoxycoumarin) from the ethanolic extract of the whole plant[16]. Later in 1977, the same research group isolated two new xanthoneslaxanthone I (1, 3-dihydroxy-6, 7-dimethoxyxanthone) and laxanthone II (1-hydroxy-3, 6-diacetoxy-7- methoxyxanthone) [16]. D. K. Bhardwaj*et al*. in 1980 carried out synthetic studies for the confirmation of the structure of laxanthone II[16].



Lacoumarin
(5-Allyloxy-7-acetoxy coumarin)

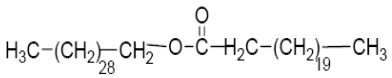


Laxanthone I
(1, 3-Dihydroxy-6, 7-dimethoxyxanthone)

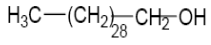


Laxanthone II
(1-Hydroxy-3, 6-diacetoxy-7- methoxyxanthone)

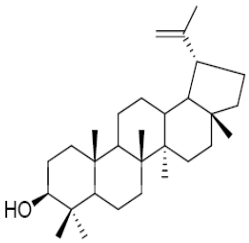
T. Chakrabortty*et al*. in the same year (1977) isolated a new aliphatic ester (*n*triaconyl*n*-tridecanoate) along with *n*-triacontanol, lupeol, 30-norlupan-3-β-ol-20-one, β-sitosterol, betulin and betulinic from the bark of *L. alba*[16].



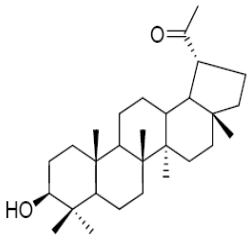
n-Triacontyl-n-tridecanoate



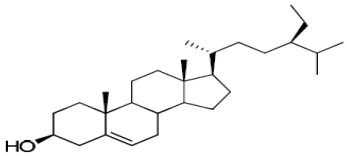
n-Triacontanol



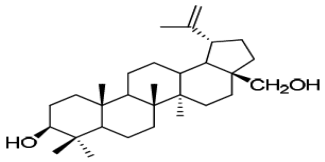
Lupeol



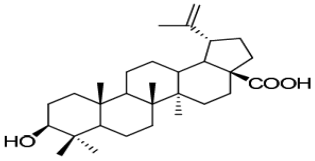
30-Norlupan-3β-ol-20-one



β-Sitosterol

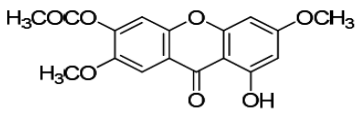


Betulin



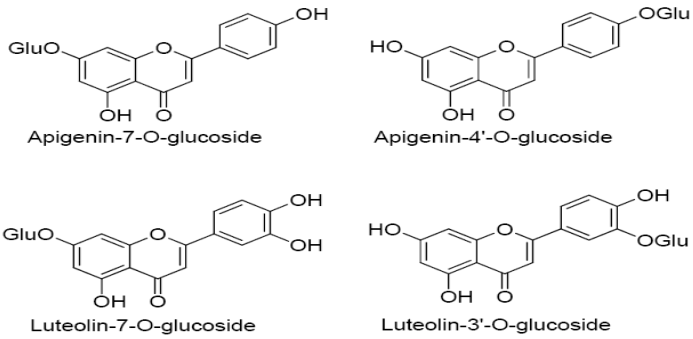
Betulinic Acid

D. K. Bhardwaj*et al*. in 1978 further reported a new xanthone named laxanthone III (1-hydroxy 3, 7-dimethoxy-6-acetoxy xanthone) from the whole plant of *L. alba*[17].

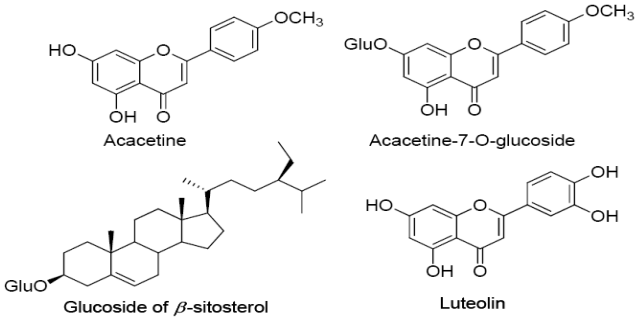


Laxanthone III
(1-Hydroxy-3, 7-dimethoxy-6-acetoxy xanthone)

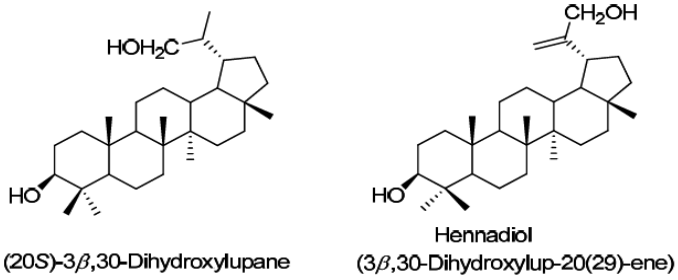
M. Afzal*et al*. in 1980 isolated four flavone glycosides apigenin-7-O-glucoside, apigenin-4'-O-glucoside with 5, 7, 4' substitution, luteolin-7-O-glucoside and luteolin-3'-Oglucoside representing 5, 7, 3', 4' substitution from the leaves of *L. alba*[17].



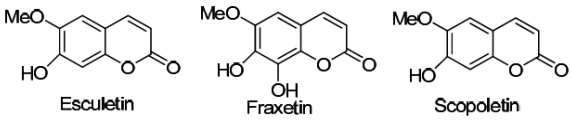
F. M. Zeinab*et al*. in the same year isolated seven crystalline compounds acacetine (Syn: Linarigenin; Linarisenin; 4'-Methoxyapigenin), acacetin-7-*O*-glucoside, glucoside of βsitosterol, luteolin, luteolin-7-*O*-glucoside, laxanthone I, laxanthone II and lawsone from the leaves of *L. alba*[17].



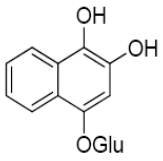
T. Chakrabarrry*et al*. in 1982 communicated two pentacyclictriterpenes from the bark of *L. alba*and elucidated their structures as (20*S*)-3β, 30-dihydroxylupane and 3β, 30-dihydroxylup-20(29)-en (hennadiol) [17].



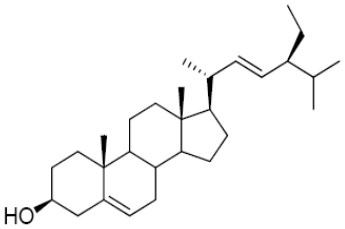
In the same year N. Nuraliev Yu and M. Kurabanov isolated three coumarins from the leaves of *L. alba*, namely esculetin, fraxetin and scopoletin[17].



In 1984 M. Afzal*et al*. proposed that lawsone may be an artifact which occurs in nature in reduced form, and during extraction under alkaline conditions is auto-oxidized to lawsone. In order to confirm they extracted the leaves with organic solvents both in the absence and presence of alkali. No lawsone could be isolated when the extraction was done in the absence of alkali while in alkaline condition the leaves extract yield lawsone. They also isolated 1, 2-dihydroxy-4-glucosyloxynaphthalene, β-sitosterol and stigmasterol from the methanolic leaves extract[17].

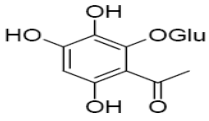


1,2-Dihydroxy-4-glucosyloxynaphthalene

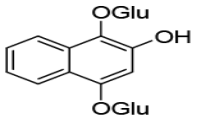


Stigmasterol

Shikhiev*et al*. in 1987 isolated D-mannitol in 0.7% yield from the stem of *L. alba*and proposed that D-mannitol could be obtained from this plant on commercial scale. D-mannitol was also found in flowers and roots of henna[18]. Y. Takeda *et al*. in 1988 isolated two novel phenolic glucosideslalioside and lawsoniaside from the ethanolic leaves extract of *L. alba* using Sephadex LH-20 and Si gel along with previously reported luteolin 3'-*O*-glucoside, luteolin 7-*O*-glucoside, 1,2-dihydroxy-4-glucosyloxy naphthalene[18].

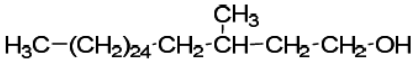


Lalioside
(2,3,4,6-Tetrahydroxyacetophenone-2-β-D-glucopyranoside)



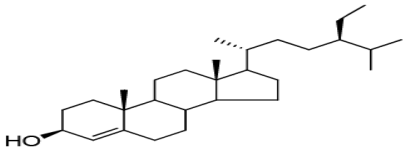
Lawsoniaside
(1,2,4-Trihydroxy-naphthalene-1,4-di-β-D-glucopyranoside)

S. Gupta *et al*. in 1992 isolated a new aliphatic hydrocarbon and characterized as 3- methylnonacosan-1-ol from the bark of *L. alba*[18].



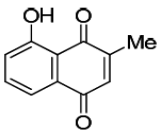
3-Methylnonacosan-1-ol

S. Gupta *et al*. in 1992 communicated a new sterol, lawsaritol and elucidated its structure as 24β-ethylcholest-4en-3β-ol from roots of *L. alba*[18].



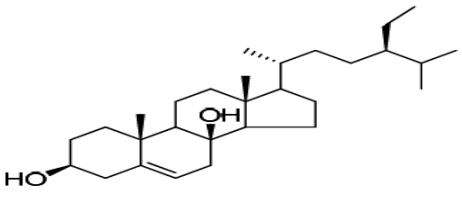
Lawsaritol
(24β-Ethylcholest-4-en-3β-ol)

S. Gupta *et al*. in 1993 isolated a naphthoquinoneisoplumbagin from the stem bark of *L. alba*and determined its structure as 2-methyl-8-hydroxy-1, 4-naphthoquinone[18].



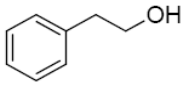
Isoplumbagin
(2-methyl-8-hydroxy-1,4-naphthoquinone)

The similar group in 1994 reported a new dihydroxysterol, lawsaritol A from the roots of *L. alba*and determined its structure on the basis of spectroscopic studies as 24β-ethylcholest-5-en-3β, 8β-diol[18].



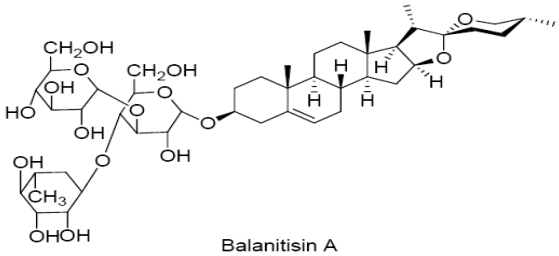
Lawsaritol A
(24β-Ethylcholest-5-en-3β, 8β-diol)

K. C. Wong *et al*. in 1995 examined the volatile components of yellow and red flowers of *Lawsoniaalba*L. by capillary GC and GC/MS. The component isolated were determined as 2-phenylethanol, β-ionone and its derivatives. Yellow flowers yielded higher percentage of volatile components as compared to red flowers[18].



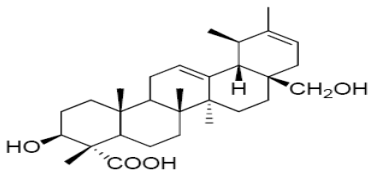
2-Phenylethanol

M. Khan *et al*. in 1996 obtained a new antiviral saponin, balanitisin A, from the fruits of *L. alba* on hydrolysis with dilute sulfuric acid it yielded diosgenin and sugars identified as 3-O-{α-D-glucopyranosyl-(1 →3)-[α-L-rhamnopyranosyl-(1→ 4)]- α-D-glucopyranoside}).

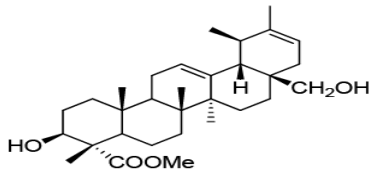


Balanitisin A
(Diosgenin 3-O-{α-D-glucopyranosyl-(1➛3)-[α-L-rhamnopyranosyl-(1➛4)]-α-D-glucopyranoside})

G. Handa*et al*. in 1997 isolated a new anticomplementarytriterpenoid, lawnermis acid from the seeds of *L. alba*along with its methyl ester[18].

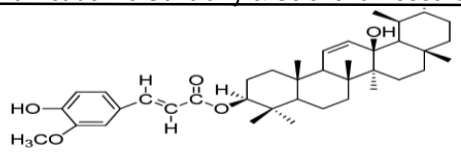


Lawnermis acid
(3β,28β-Dihydroxy-urs-12,20-dien-23-oic acid)

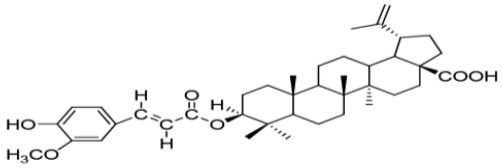


Methyl ester of lawnermis acid

B. S. Siddiqui*et al*. in 2001 isolated two new pentacyclictriterpenoids, lawsonin and lawsonic acid from the aerial parts of *L. alba* and deduced their structures through spectroscopic studies as 3α-*E*-ferulyloxy-urs-11-en-13β-ol and 3α-*E*-ferulyloxy-lup-20(29)-en-28-oic acid respectively [19].

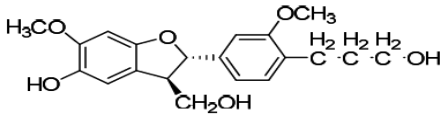


Lawsonin
3α-*E*-Ferulyloxy-urs-11-en-13β-ol

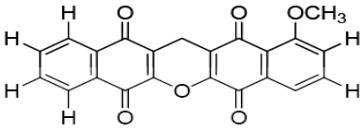


Lawsonic acid
3α-*E*-Ferulyloxy-lup-20(29)-en-28-oic acid

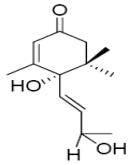
The same group in 2003 reported two new compounds, lawsonicin and lawsonadeem together with a hitherto unreported compound vomifoliol from the aerial parts of *L. alba*. Their structures were elucidated as 2,3-dihydro-5-hydroxy-3-(hydroxymethyl)-2-[4-(3-hydroxypropyl-3-methoxyphenyl]-6-methoxy-1-benzofuran (lawsonicin), 1-methoxy-13Hdibenzo[b,i]xanthenes-5,7,12,14-tetrone (lawsonadeem) and (4S)-4-hydroxy-4-[(1E,3R)-3-hydroxybut-1-enyl]-3,5,5-trimethylcyclohex-2-1-one (vomifoliol) by spectroscopic techniques and chemical transformations[19]. The structure of lawsonicin was later on revised on the basis of its synthesis as dihydrodehydrodiconiferyl alcohol.



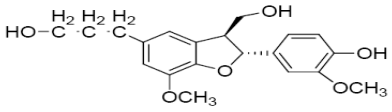
Lawsonicin
(2,3-Dihydro-5-hydroxy-3-(hydroxymethyl)-2-[4-(3-hydroxypropyl-3-methoxyphenlyl]-6-methoxy-1-benzofuran)



Lawsonadeem
(1-Methoxy-13H-dibenzo[b,i]xanthenes-5,7,12,14-tetrone)

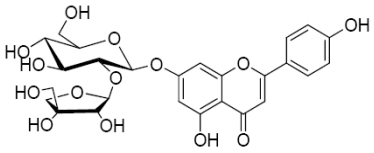


Vomifoliol
(4S)-4-Hydroxy-4-[(1*E*,3*R*)-3-hydroxybut-1-enyl]-3,5,5-trimethylcyclohex-2-1-one

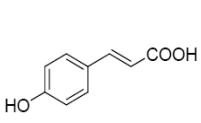


Revised structure of lawsonicin
Dihydrodehydrodiconiferyl alchol

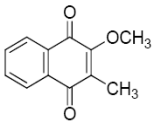
Michaeil B. R. *et al*. in 2004 isolated three new source constituents from the methanolic leaves extract of *L. alba* namely apiin, *p*-coumaric acid and -methoxy-3-methyl-1,4-napthoquinone, together with formerly reported constituents apigenin, cosmosiin (apigenin-7-glycoside), lawsone and luteolin[19].



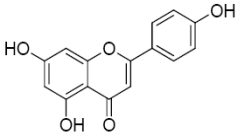
Apiin



p-Coumaric acid

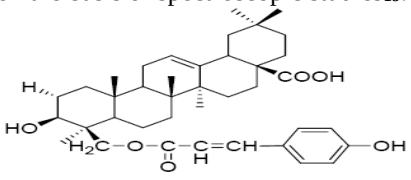


2-Methoxy-3-methyl-1,4-napthoquinone

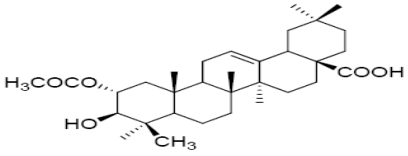


Apigenin

B. S. Siddiqui*et al*. in 2005 further isolated two new triterpenoidslawsowaseem and lawsoshamim from the aerial parts of *L. alba* and elucidated their structure as 3β -hydroxy-24-*p*-E-coumaroyloxy-olean-12-en-28-oic acid and 2-acetoxy-3β -hydroxy-olean-12-en-28-oic acid on the basis of spectroscopic studies[19].

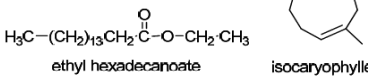


Lawsowaseem
(3β -Hydroxy-24-*p*-*E*-coumaroyloxy-olean-12-en-28-oic acid

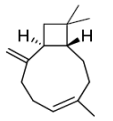


Lawsoshamim
2-Acetoxy-3*β*-hydroxy-olean-12-en-28-oic acid

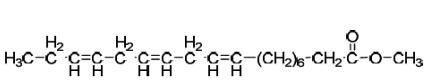
A.O. Yedeji*et al*. in 2005 analyzed the leaves of *L. alba*L. (henna) by GC and GC/MS and recognized thirty six constituents including ethyl hexadecanoate, isocaryophyllene, (*E*)-*β*-ionone, methyl linolenate and methyl-(*E*)-cinnamate[19].



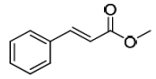
ethyl hexadecanoate



isocaryophyllene

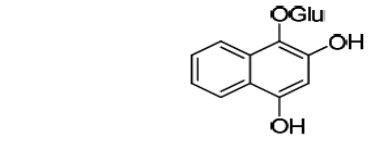


methyl linolenate

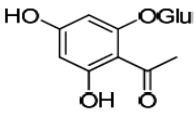


methyl-(*E*)-cinnamate

A. B. Hsouna*et al*. recently isolated five phenolic glycosides from the *n*-butanol fraction of the leaves of *L. alba* including one new constituent characterized as 1,2,4- trihydroxynaphthalene-1-*O*-*β*-D-glucopyranoside, a new source compound 2,4,6-trihydroxyacetophenone-2-*O*-*β*-D-glucopyranoside and three known compounds, luteolin-7-*O*-*β*-D-glucopyranoside, lalioside (2,3,4,6-tetrahydroxyacetophenone-2-*O*-*β*-Dglucopyranoside) and lawsoniaside (1,2,4 trihydroxynaphthalene-1,4-di-*O*-*β*-Dglucopyranoside).



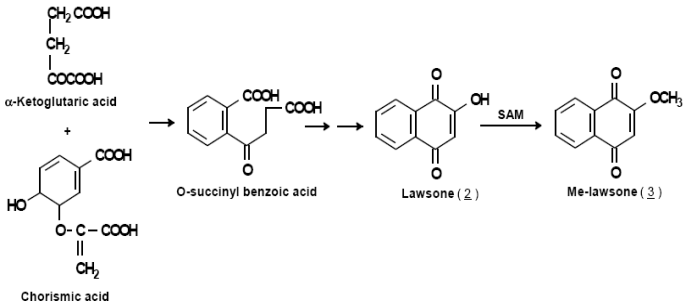
1,2,4-Trihydroxynaphthalene-1-O-β-D-glucopyranoside



2,4,6-Trihydroxyacetophenone-2-O-β-D-glucopyranoside

2.7 Biosynthesis Of Lawson

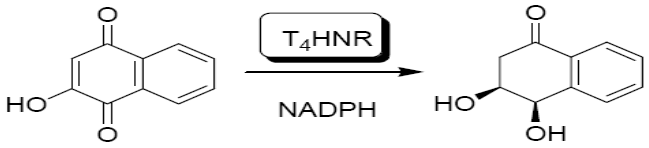
Lawsone (2-hydroxy-1,4-naphthoquinone 2) and Me-lawsone (2-methoxy-1,4-naphthoquinone 3) are the two main antifungal naphthoquinones found naturally in *Impateinsbalsamina*L. Biosynthetically, it has been proposed based on feeding experiments that lawsone is formed in plant *via* 2-succinylbenzoate, a key intermediate arise from glutamate and chorismate (scheme 1). However, none of the enzymes involved in the formation of the naphthoquinones have been found in plants. This prompted us to investigate the biosynthetic pathway of lawsone and its derivative Me-lawsone. We first examined for a suitable enzyme source by establishing various types *in vitro* cultures of *I. balsamina*cultures. It was found that the root cultures of *I.balsamina*could produce a number of natural products, mostly belonging to the chemical groups of coumarins and napthoquinones including lawsone and Me-lawsone. This indicated that the biosynthetic pathways of both naphthoquinones and coumarins were actively operated in the root culture and thus it was suitable for being used for studying the biosynthetic enzymes involved in the formation of these compounds.



Scheme1. Proposed Biosynthetic pathway of lawsone in Impatienbalsamina.

2.7.1 Lawsone and Enzymatic Reduction

Lawsone, is a red-orange dye present in the leaves of the henna plant (*Lawsoniainermis*). Humans have used henna extracts containing lawsone as hair and skin pigments for more than 5000 years. We have used this natural Product as a substrate, which on reduction catalyzed by the enzyme called tetrahydroxynaphthalenereductase (T4HNR) from *Magnaprothegrisea*, gave *cis*-ketodiol with high yield (95%) and high *ee*(99%).

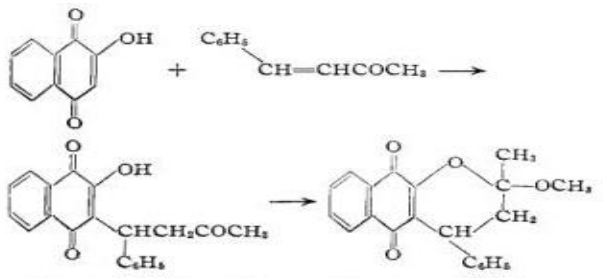


Lawsone *cis*-keto-diol

Lawsone was also used extensively to study the mechanism of T4HNR-catalyzed reduction that has led to the formation of a *cis*-keto-diol.

2.7.2 A Michael Reaction of Lawson

In connection with the preparation of various 3-substituted-2-hydroxy 1, 4-naphtoquinones for the application to antimalarial studies, the present author found that lawsone would react with benzalacetonein pyridine to give an addition product resulting from a Michael type reaction. In this respect, lawsone is similar in reactivity to 4-hydroxycoumarin which likewise has been found to add to various α,β-unsaturated ketones. The structure of the product was demonstrated by analysis and by ring-closure to a cyclic ketal. The two reactions are summarized as:



Since both the quinone adduct and its cyclization product were found to be entirely inactive against *p.lophurae* in ducks, no extension of this reaction to other α,β-unsaturated ketones was attempted.

2.8 Henna and Tannin

Plant tannins, such as found in henna, react with collagen and keratin and preserve protein structures in skin and leather, keeping them supple, resistant to desiccation and degradation[13]. Henna stains protect skin by packing the "band" regions of the fibrils with tannin, which prevents them from separating, thus preserving the macro-molecular structure and slowing the spread of decay or disease[13]. Plant tannins preserve mummies and bodies in bogs from desiccation or decay. On living people, henna's tannin keeps skin, hair and nails supple, deterring drying and cracking in arid climates. Henna stains also block damaging UV sunlight, a serious threat to skin health in the latitudes where henna is used. Some medical studies have tested folk remedies that include henna, and found henna is effective against ringworm[14]. Henna's reputation as having "*baraka*", blessedness, the ability to deter the Evil Eye, may be associated these beneficial characteristics.

2.9 Black Henna or Kali Mehndi

Henna is not black. It does not cause blisters and open sores. paraphenylenediamine (PPD) black hair dye can cause blisters and sores.There are several things marketed as "Black Henna", and some things believed to be "Black Henna". Some are very dangerous. Some are harmless. When paraphenylenediamine is used to make black temporary tattoos, often called "black henna", it can cause blistering, open sores, scarring, and lifelong health problems**.** The black henna that is available in the market is not natural henna. It is obtained from the indigo plant and also contains the chemical paraphenylenediamine or PPD. Sometimes these are added to henna to produce black henna. Black henna is extremely dangerous to health since it is a transdermal toxin and a potential carcinogen apart from the fact that it causes allergy in many of those who use black henna. Getting a black henna tattoo makes the hair sensitive to hair dyes and other chemicals and if a person has had a black henna tattoo and follows it up later with a chemical hair dye, it could even cause a life threatening allergic reaction. A plant known as "wasma" was also mixed with henna to dye hair black. If you see a package of "black henna" in a Middle Eastern or Indian grocery it is probably indigo. If you see a package of black hair dye from an American cosmetic company, it has some form of para-phenylenediamine in it.

Para-Phenylenediamine (PPD) is an aromatic amine compound; its chemical formula is $C_6H_8N_2$ and its molecular weight is 108.15 g/mol. It is white to light purple powder that darkens on exposure to air (it oxidizes, turning first red, then brown then finally black); it is slightly soluble in water[23]. It is primarily used as an ingredient of oxidative hair coloring products at a maximal concentration of 4.0%, however, after mixing in a 1:1 ratio with hydrogen peroxide prior to use this concentration will be 2% at the time of application to the hair. In addition to hair dyes, PPD may also be found in fur or textile dyes[23]. *para*-Phenylenediamine is also used as a photographic developing agent as well as an antioxidant in rubber compounds. It has also been used to intensify the color of henna and to accelerate the dyeing process. Accidental or deliberate ingestion of henna containing PPD has a high mortality rate (up to 31%) owing to rhabdomyolysis and renal failure. Individuals may be occupationally exposed to PPD during its manufacture or use, and the exposure may occur through inhalation, skin and/or eye contact, and ingestion[23].short-term exposure to high

levels of PPD (acute effects) may cause severe dermatitis, eye irritation and tearing, asthma, gastritis, renal failure, vertigo, tremors, convulsions, and coma in humans. Eczematous contact dermatitis may result from long-term exposure (chronic effect) in humans[23]. According to Scientific Committee on Consumer Products (SCCP), *para*-phenylenediamine is a very strong potential skin sensitizer and it is included as such in theEuropean Standard Series for diagnostic patch testing for eczema patients[24]. *para*-Phenylenediamine (PPD) is an allergen; even if someone does not have a reaction the first time they are exposed to it, they can become "sensitized" to PPD over time and can have adverse reaction upon re-exposure[24].

In addition, PPD provokes cross-allergy, making people allergic to other substances which contain *para*-substituted amino compounds[24]. No information is available on the reproductive, developmental, or carcinogenic effects of PPD in humans. However, SCCP reported that PPD together with hydrogen peroxide may be carcinogenic according to experimental studies with rats[25].Recently *para*-phenylenediamine has been mixed with natural henna to give an ebony color (black henna) instead of the orange/reddish color given by natural henna. The other reason for adding PPD to the natural henna is to speed up (shorten the time) of the tattooing process, while natural henna staining takes 4 to 12 hours, addition of PPD can reduce this time to an hour or two and also there will be a longer lasting effect as well. Thus, a new pattern of exposure to PPD has been recognized through henna art which increases the risk of developing adverse health effects related to PPD[25]. Acute allergic contact dermatitis, eczema, chemical burn, acute renal failure, acute and severe angioneuroticedema, abdominal pain and vomiting as adverse health effects associated with the use of henna containing PPD (black henna) are well documented in the literature[25]. In addition, cases of persons being sensitized from use of black henna (containing PPD) followed by cross reaction to oxidative hair dyes and to clothing dyes have also been described in the literature[25-26]. Always use safe, natural red-brown henna in your henna work. Never use any "black henna" product containing para-phenylenediamine to stain skin.

3. EXPERIMENTAL

3.1 Sample Collection and Preparation

Powder of dried henna leaves, *Lawsoniainermis* material (40gm) will be collected from Amhara region North Gondar and will be stored in air sealed brown bottles at 4^{o}C until used for the extraction.

3.2 Extraction Method

Powder of dried henna leaves (40 g) is placed in a large beaker and distilled water (2 L) is added together with a magnetic stirring rod. The suspension is stirred on a magnetic stirrer with heating while the temperature is kept at 70 °C. After 45 min, the colour of the green suspension turns to brown. The increasing content of lawsone in the aqueous phase can be seen by TLC on standard silica gel plates with the eluent methanol-ethyl acetate (1:2, v/v) + 0.5% acetic acid. The Rf of the dark orange lawsone spot is 0.6. After 4 h, solid NaHCO3 (8.4 g) is added. The suspension is filtered by gravity overnight over three large glass funnels with filter paper (diameter 30 cm). This kind of fi ltration is slow but works reliably. Attempts to force the pace by suction filtration are not advisable because then colloidal particles will rapidly plug the pores of the filter. The filtrates are combined and acidified to pH 3 by addition of 0.12 M HCl. The brown extract undergoes a clarification in this step and turns slightly cloudy. The swollen plant material is discarded. The filtrate is extracted with diethyl ether (4 × 200 mL). In the final extraction, the ether turns to a very pale yellow, indicating the end of extraction. The aqueous phase does not change its brown color during extraction but turns clear and can be discarded after the extraction. The combined ethereal phases are washed with water (3 × 50 mL) and dried over MgSO4. The ether is removed completely in vacuo to leave a reddish brown solid (660 mg) as crude product.

3.3 Purification

The crude lawsone is purified by column chromatography. Conditions (not optimized): column 40 × 3 cm; stationary phase, silica gel 60 (45 g, 0.040–0.063 mm); eluent, ethanol-ethyl acetate (1:2, v/v) (by using ethanol instead of methanol, any dissolution of tiny silica gel particles is suppressed). The crude product is dissolved in 10 mL of the eluent, placed on the top of the column and the elution is started. It is easily observable by the different colored zones formed. Fractions of 10 mL are taken; in the region of the lawsone zone the fraction size is reduced to 3mL. The composition of all fractions is checked by TLC.

3.4 Structural Elucidation

The structure of the pure component(s) obtained with appropriate amount will be determined by using spectroscopic methods like UV-VIS, IR, MS, NMR and by correlation with previously identified compounds from the species.

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